Co-extraction of RNA and DNA from plant tissue

Dominik Buchner

University of Duisburg-Essen, Aquatic Ecosystem Research

ABSTRACT

This protocol describes how to co-extract RNA and DNA from plant tissue samples. Samples are homogenized and simultaneously lysed by bead-beating. Cell debris is then caught with a pre-filter column, the DNA is then subsequently bound to a silica column, while the RNA passes the membrane. The RNA in the flow-through is then precipitated with 100% ethanol and bound to a second silica column. Both, DNA and RNA are washed with different wash buffers to remove remaining proteins and other contaminants and finally eluted in separate tubes. If the user is just interested in the RNA, the DNA spin-column can just be discarded.

GUIDELINES

Follow general lab etiquette. Wear gloves to prevent contamination of samples. Clean the workspace before starting and after finishing with 80% EtOH.

MATERIALS

Materials required:

Below all materials needed for the protocol are listed. Vendors and part numbers are listed but interchangeable depending on the supply situation.

Chemicals:

- Guanidinium thiocyanate
- Tris ultrapure 99.9%
- Hydrochloric acid fuming 37%

Pre-filter columns

License: This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Protocol status: Working

We use this protocol and it's working

Created: Feb 08, 2023

Last Modified: Feb 10, 2023
**PROTOCOL integer ID:** 76687

- Guanidine hydrochloride Fisher Scientific Catalog #10543325
- Ethanol absolute 99.8% Fisher Scientific Catalog #11994041

**Labware:**
- 2 mL screwcap tubes Sarstedt Catalog #72.693
- 2 mm zirconia beads BioSpec Products Catalog #11079124zx
- Glass Beads 0.1 mm dia BioSpec Products Catalog #11079101
- EconoSpin mini spin column Epoch Life Science Catalog #1920-050

**Stock solutions:**

1. **1 L Tris stock solution [M] 1 Molarity (m) pH 7.5**
   - Add 121.1 g Tris ultrapure 99.9% to a beaker
   - Adjust volume to 800 mL with ddH$_2$O
   - Adjust pH to 7.5 with HCl
   - Adjust volume to 1 L with ddH$_2$O

2. **1 L sodium chloride stock solution [M] 5 Molarity (m)**
   - Add 292.2 g sodium chloride to a beaker
   - Adjust volume to 1 L with ddH$_2$O
   - Sterilize by filtering and store at Room temperature

3. **1 L Tris stock solution [M] 1 Molarity (m) pH 8.5**
   - Add 121.1 g Tris ultrapure 99.9% to a beaker
   - Adjust volume to 800 mL with ddH$_2$O
   - Adjust pH to 8.5 with HCl
   - Adjust volume to 1 L with ddH$_2$O

4. **1 L DNA wash buffer 2 stock solution [M] 50 millimolar (mM) Tris pH 7.5**
   - Add 50 mL of [M] 1 Molarity (m) Tris stock solution to a beaker
   - Adjust volume to 1 L with ddH$_2$O
Sterilize by filtering and store at Room temperature

**Working solutions:**

1 L GITC lysis buffer
- [4 Molarity (m) Guanidinium thiocyanate](#)
- [10 millimolar (mM) Tris](#)
  - Add 472.6 g guanidinium thiocyanate to a beaker
  - Add 10 mL of 1 Molarity (m) Tris stock solution
  - Adjust volume to 1 L with ddH₂O
  - Stir until the GITC is completely dissolved (heating will speed this up)
  - Sterilize by filtering and store at Room temperature

1 L RNA wash buffer 1
- [900 millimolar (mM) Guanidinium thiocyanate](#)
- [10 millimolar (mM) Tris](#)
- [20 % (v/v) Ethanol absolute](#)
  - Add 106.3 g guanidinium thiocyanate to a beaker
  - Add 10 mL of 1 Molarity (m) Tris stock solution
  - Add 200 mL Ethanol absolute
  - Adjust volume to 1 L with ddH₂O
  - Sterilize by filtering and store at Room temperature

1 L RNA wash buffer 2
- [100 millimolar (mM) sodium chloride](#)
- [10 millimolar (mM) Tris](#)
- [80 % (v/v) ethanol absolute](#)
  - Add 20 mL of 5 Molarity (m) sodium chloride stock solution
  - Add 10 mL of 1 Molarity (m) Tris stock solution
  - Adjust volume to 200 mL with ddH₂O
  - Adjust volume to 1 L with ethanol absolute
  - Sterilize by filtering and store at Room temperature

1 L DNA wash buffer 1
- [2.5 Molarity (m) Guanidinium chloride](#)
- [10 millimolar (mM) Tris](#)
- [57 % (v/v) Ethanol absolute](#)
  - Add 238.9 g guanidinium chloride to a beaker
  - Add 10 mL of 1 Molarity (m) Tris stock solution
  - Adjust volume to 430 mL with ddH₂O to dissolve the GuHCl
  - Adjust volume to 1 L with Ethanol absolute
  - Sterilize by filtering and store at Room temperature

---

[https://dx.doi.org/10.17504/protocols.io.n2bvi8qxngk5/v1](https://dx.doi.org/10.17504/protocols.io.n2bvi8qxngk5/v1)

Oct 10 2023
1 L DNA wash buffer 2 (10 millimolar (mM) Tris, 80 % (v/v) ethanol absolute) 7.5
- Add 200 mL DNA wash buffer 2 stock solution to a beaker
- Adjust volume to 1 L with Ethanol absolute
- Sterilize by filtering and store at Room temperature

1 L elution buffer (10 millimolar (mM) Tris 8.5)
- Add 10 mL of 1 Molarity (m) Tris stock solution 8.5 to a beaker
- Adjust the volume to 1 L with ddH₂O
- Sterilize by filtering and store at Room temperature

SAFETY WARNINGS

Buffers containing guanidine produce highly reactive compounds when mixed with bleach. Don't mix the extraction waste with bleach or solutions that contain bleach. Reagents are potentially damaging to the environment. Dispose waste as mandated.

BEFORE START INSTRUCTIONS

Make sure all buffers are prepared before starting.

Sample preparation and lysis

1 For each sample prepare one 2 mL screwcap tube pre-filled with approximately 400 mg of 2 mm zirconia beads and 0.1 mm glass beads.

2 Add up to 200 mg of plant tissue to the prepared tube.
3. Add **800 µL** GITC lysis buffer to the sample tube.

4. Immediately bead beat for **00:05:00** at maximum speed.

5. **Room temperature, 00:00:10 , at maximum speed**

6. Transfer **700 µL** of the crude lysate to a pre-filter column.

---

**Note**

For samples with a high RNA content less starting material might lead to better results. For most sample types **50 mg** of starting material will yield a sufficient amount of DNA and RNA for downstream analysis.

**Note**

For complete inactivation and destruction RNAses of 2-Mercaptoethanol can be added in addition. We usually don't because then the samples have to be handled under a fume hood until all lysate has been handled and discarded appropriately.

**Note**

Depending on the bead beater used in this step the time might have to be adjusted. We'd recommend to bead beat the sample until the material is completely homogenized.
DNA binding

8 Transfer **700 µL** of the flowthrough from step 7 to a silica spin column to bind the DNA in the lysate. *Keep the flow-through. Mark the spin column as the DNA column.*

**Note**

The protocol will work with all kinds of silica spin columns. See materials section for what we use.

RNA precipitation and binding

9 Add **350 µL Ethanol absolute** to the flow-through from step 8 to adjust the binding conditions to bind RNA to the silica column.

10 Vortex the samples to mix the lysate with the ethanol. Do not centrifuge.

11 Load the mixture on a second spin column. *Mark this column as the RNA spin column.*

**11000 x g, Room temperature, 00:00:15** and discard the flow-through.

**Note**

Two loading steps will be necessary to pass the complete volume through the spin column.

Washing steps
12. Add **700 µL RNA wash buffer 1** to the RNA spin column, **11000 x g, Room temperature, 00:00:15** and discard the flow-through.

13. Add **500 µL RNA wash buffer 2** to the RNA spin column, add **500 µL DNA wash buffer 1** to the DNA spin column, **11000 rpm, Room temperature, 00:00:15** and discard the flow-through.

14. Add **500 µL RNA wash buffer 2** to the RNA spin column, add **500 µL DNA wash buffer 2** to the DNA spin column, **11000 rpm, Room temperature, 00:00:15** and discard the flow-through.

**Column drying and elution**

15. **11.000 rpm, Room temperature, 00:01:00** to dry the silica membrane of the spin columns. Transfer the spin column to a fresh 1.5 mL microcentrifuge tube.

16. Add **100 µL elution buffer** directly to the silica membrane. Incubate the column for **00:03:00** at **Room temperature**

17. **11.000 rpm, Room temperature, 00:01:00**, store the eluted RNA at **-80 °C** and the eluted DNA at **-20 °C**
Expected result of the described protocol. Extraction was carried out in 4 replicates, left part of the gel picture shows the DNA fraction of the sample, while the right part shows the RNA fraction.